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(54) **NMR analysis of fat content in mixtures, using chemical drying**

(57) Determination of the fat or oil content of substances with high water content, in particular of food, is accomplished with a low resolution NMR spectrometer. Prior to the NMR measurement, the substance is mixed with a chemical drying agent, preferably with CaO powder. The protons originally in free water are then rigidly bound and their short T2 time permits discrimination.
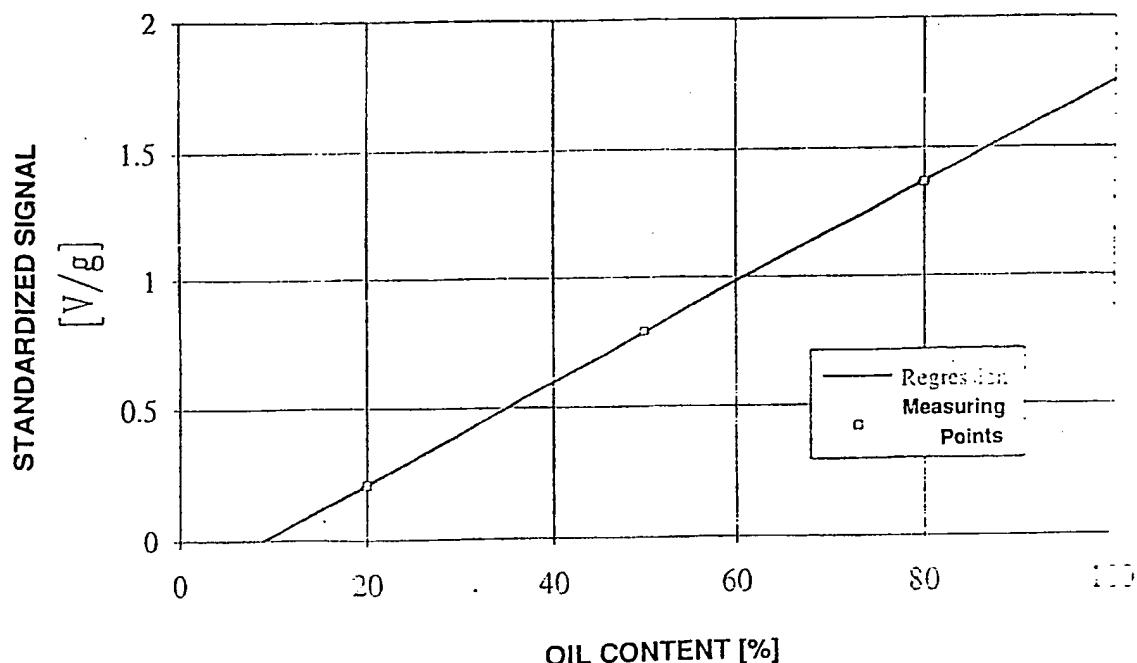
Fig. 2

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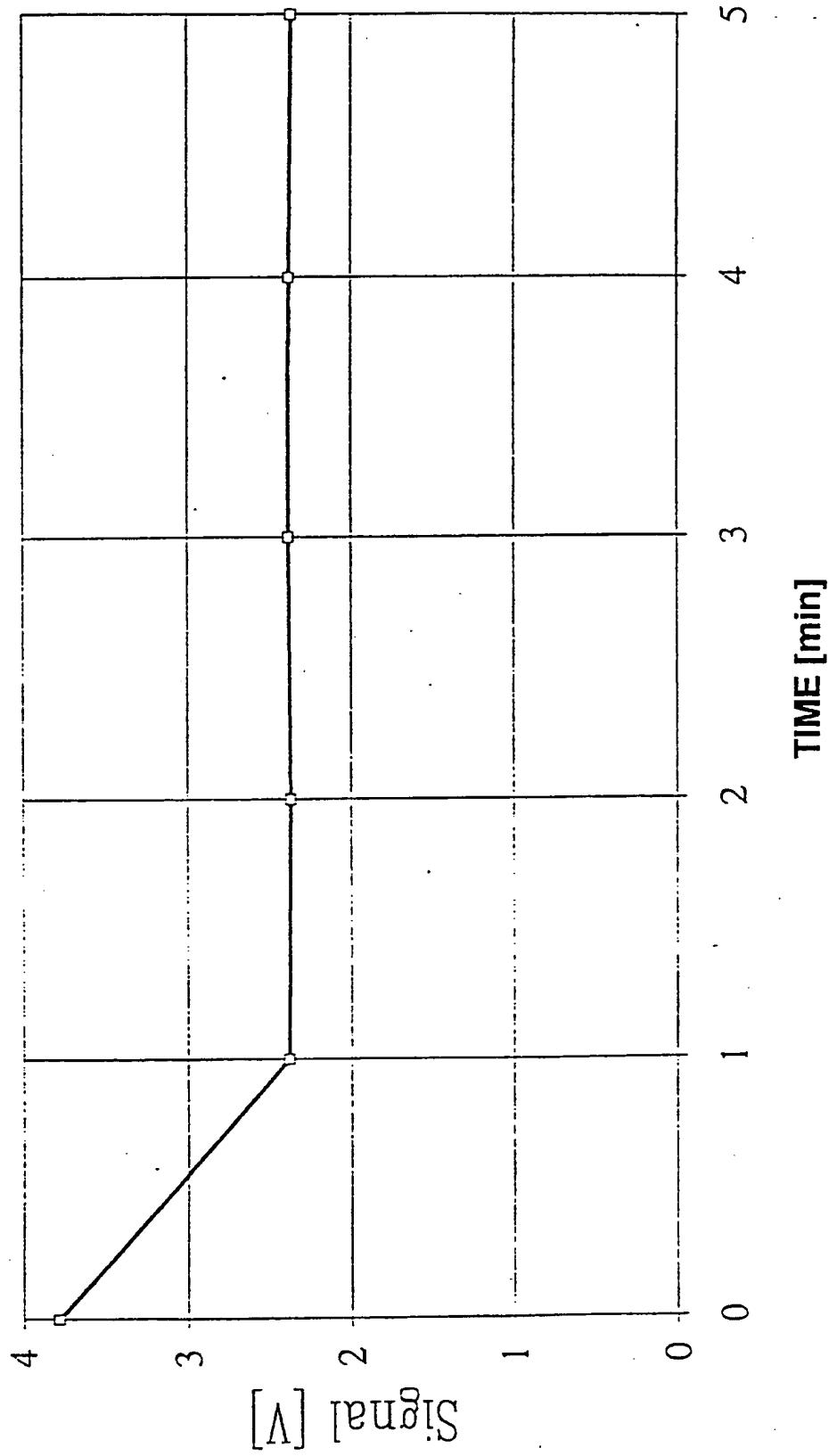
Fig. 1

Fig. 2
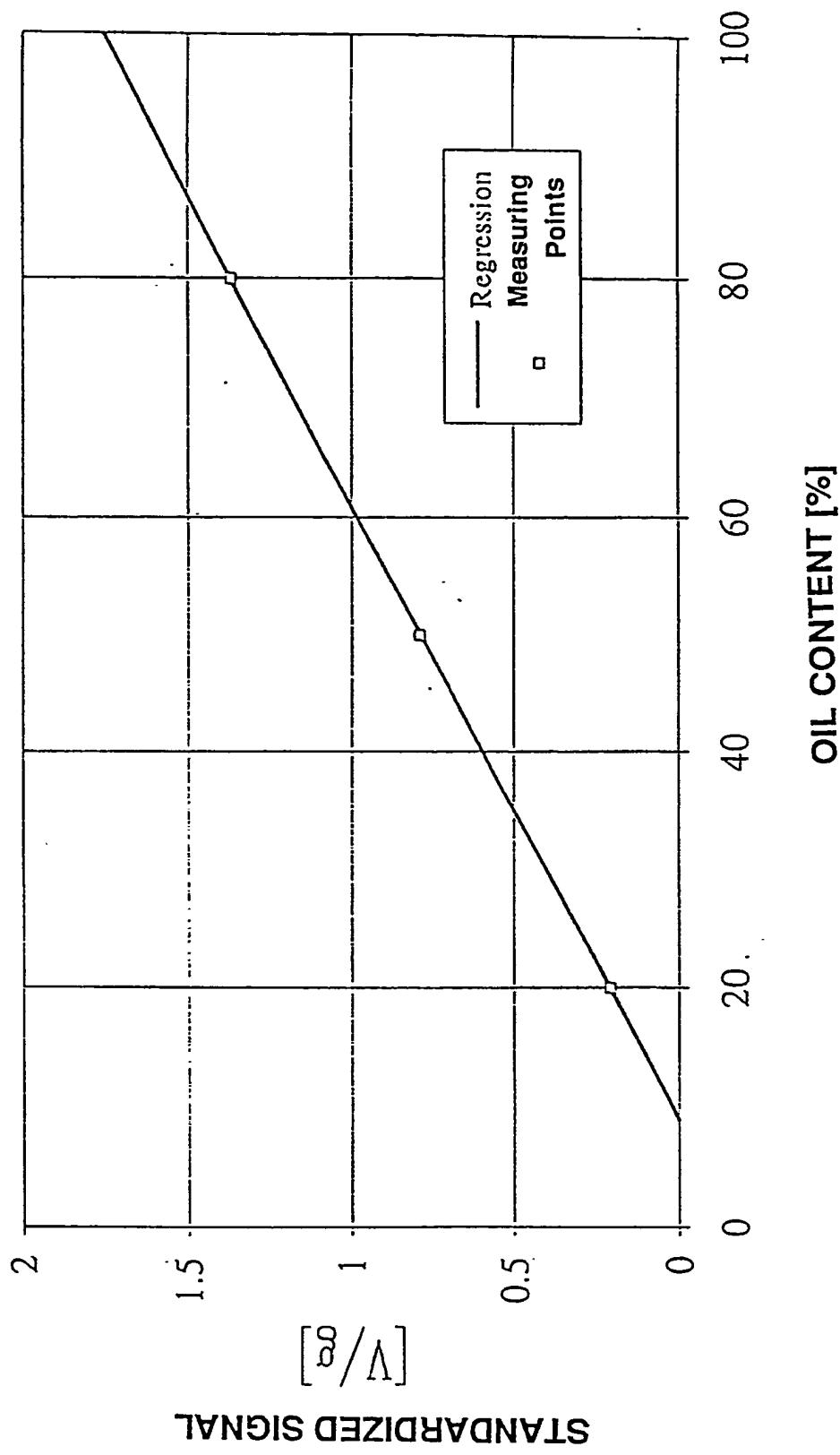
Method of Determining Fat or Oil Content

The invention concerns a method to determine the fat or oil content of substances with high water content, in particular of food, by means of an NMR spectrometer.

Such a method is for example known from the article by H.Weisser in Zeitschrift für Lebensmitteltechnologie 28 (1977) 3, pages 97-101.

The determination of the fat content by means of a nuclear resonance pulse spectrometer of low spectral resolution with a magnetic field strength of maximum about 1 T and an average homogeneity of about 10^{-5} across the sample volume and as a result with a proton resonance frequency lower than about 50 MHz, e.g. the "MINISPEC" of the company BRUKER, is not possible with products with a water content of, for example, more than 13 % because of the overlap of water and fat signals, since at a higher water content the contribution of the water to the total signal increases highly overproportionally. Thus, determination of such products in their native state by means of nuclear resonance is limited to high resolution devices, which are, however, much too expensive for this purpose alone.

For low moisture contents in the range of a few percent a low resolution device, too, yields reliable results, since in this case the contribution of the water signal has practically completely died away after about one millisecond and as a result it is possible to measure only the fat or oil content, respectively. By combination of two measurements at

different times after the excitation pulse it is even
possible, also to measure the moisture content
quantitatively apart from the fat content (see the articles
of S.A.Jones in BRUKER Minispec Application Note 17 (1)

5   1983, P.J.Barker in BRUKER Minispec Application Note 32 (2)
1986 and P.N.Tiwari and W.Burg in J.American Oil Chemist's
SOC. 57, No. 3, pages 119-121 (1979)). However, this
method fails with larger water contents, since these have
spin-spin relaxation times T_2 comparable to those of the

10   fat content and therefore the signal contributions can no
longer be practically separated.

In order to perform such determinations with a low
resolution device, it has been necessary hitherto to

15   eliminate the water by pre-drying in an oven, for example
in a drying oven, a vacuum oven, a microwave oven or the
like. The need for such drying apparatus and the time
needed are both disadvantages of this method.

20   In accordance with the present invention, water is removed
from such a sample at least partly by means of chemical
drying.

By the chemical removal of the water, and as a consequence,
25   the overlapping water signal, the signal caused by the fat
can be measured quickly,

easily and unambiguously. In this respect it is ir-
relevant whether the water has been removed com-
pletely. It is only important that the water con-
tent after the chemical drying is below a given
threshold. This threshold depends on the fraction
of water which is rigidly bound inside the sub-
stance. Experimentally, about 13 % has been found
to be a usable threshold.

A preferred chemical drying agent should fulfill
the following criteria as far as possible:

1. Neither it nor its reaction product should
    give any NMR signal within the measuring range.

2. Both it and its reaction product should not be
    poisonous and should be tolerable for the
    environment.

3. It should quickly bind as much water as
    possible.

4. The reaction heat released must not cause any
    danger for the personnel.

5. It should be inexpensive.

A chemical which largely shows these qualities is
calcium carbide. The reaction product is the easily
inflammable acetylene. Therefore one should work
under a flue if using calcium carbide. This is also
required for the additional reason that due to
impurities there is the danger that in addition to
acetylene PH_3 is also formed, which at the least

has an unpleasant smell and which is also poisonous
in higher concentrations.

In a particularly preferred method calcium oxide
5   (CaO) is used as a drying agent. This substance
fulfills all five requirements without the
disadvantages of   calcium carbide.

The basic reaction with water may be expressed as
10   follows:

$$CaO + H_2O \rightarrow Ca(OH)_2$$
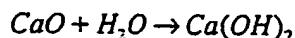
The heat released during this reaction is not
15   sufficient to be dangerous. But it is sufficient to
considerably heat up a sample of average water
content starting from room temperature so that, if
required, only a short time of tempering is needed
in order to reach the necessary measuring tempera-
20   ture for the determination. This temperature is
usually 40°C.

The invention is explained in detail with reference
to the following example.
25

Obviously, the features that have been described above
and will be explained hereafter can be used not only
in the described combination but also in any other
combination or individually without leaving the scope
30   of the present invention.

Usually, in a first step the spectrometer is cali-
brated by means of substances of known fat content.
35   The time schedule and quite generally the measuring
parameters should largely be identical to those of

the subsequent measurement. The following procedure
has proved to be advantageous. A glass tube of for
example 13 mm length is filled to just below half
its length with the calibration sample of known fat con-
5   tent and the weight of the contents is recorded.
CaO powder of about the same mass is added so that
the tube is not quite filled. This ratio of masses
has turned out to be appropriate for a wide range
of mayonnaise samples with water contents of 17 %
10   to 65 %. The amount of added CaO must be adapted to
the maximum expected water content (e.g. 65 %) and
should be chosen such that the corresponding amount
of water can be completely converted together with
the CaO. A surplus of CaO is on its own not harmful
15   but only worsens the filling factor of the measure-
ment. However, the CaO content must at least be
sufficient to reduce the water content after the
reaction below the threshold of for example 13 %.

20   After the reaction with water the total volume in-
creases because of the lower density of calcium
hydroxide so that the standard filling level of
about 30 mm is approximately reached. The mixture
is carefully stirred with a spatula. Material still
25   sticking to the spatula is wiped off with
paper. The paper should be dry so that in the
measuring range no additional water signal is pro-
duced. The paper is pressed onto the sample inside
the sample tube and stays inside the tube. After
30   this, the sample has heated up noticeably and is
only brought to measuring temperature in a short
additional tempering step. However, this tempering
step can also be omitted.

35   The proton NMR signal is then measured and
correlated by means of a table to the known fat

content. This calibration procedure is performed with several (in general at least three) calibration substances. This yields a correlation between NMR signal, standardized to the mass of the calibration sample, and the fat content which has to be interpolated inside the range of interest. This correlation is stored in the computer of the spectrometer, e.g. in the form of a table or analytically. It should be emphasized that the calibration, assuming a sufficient amount of CaO, is independent of the water contents of the calibration samples, just as the eventual measurements should be independent of the water contents of the measuring samples.

A calibration, once established, can be used for a long time, as long as nothing changes with respect to the measuring procedure, to the apparatus, and to boundary conditions. It goes without saying that the calibration should periodically be checked.

During the subsequent measurement of unknown products one has to proceed in the same way. However, the measured NMR signal, standardized to the sample mass, is compared to stored calibration tables or calibration curves, respectively, and from this a fat content is derived which is displayed.

The measuring sequence for the calibration and the measurement is usually a spin echo sequence, i.e. an excitation high frequency pulse (generally a 90° pulse) is after a delay period in the millisecond range, e.g. after three milliseconds, followed by a second pulse usually a 180° pulse phase shifted by 90°. After again the same delay time of e.g. further three milliseconds a spin echo can be detected. For this purpose

it is sufficient to take one measuring point at the
known point of time of the occurence of the spin
echo. Details of the pulse sequences may be found
in textbooks on nuclear resonance. A spectrometer
with which the invention can be performed is
described in the brochure "Minispec PC 100"
(September 1990) of the company BRUKER Analytische
Meßtechnik GmbH. To improve the signal-to-noise
ratio several echoes can be produced and accumula-
ted by repeated 180° pulses, or the
measurement can be repeated and accumulated after a
delay time which is large compared to the maximum
spin-lattice relaxation time of the sample (several
seconds). The spin echo method is
particularly advantageous since at the point of
time of the echo, e.g. after six milliseconds, the
signal contributions of rigidly bound water
molecules, e.g. in lipid form, with correspondingly
short spin-spin relaxation times T_2 are already
decayed. Obviously after the drying procedure
leading down to a water content threshold of e.g.
13 % and below, predominantly bound H_2O molecules
remain so that the contribution of the water signal
to the spin-echo signal has decayed
overproportionally to a negligible value. This
result has been confirmed experimentally for a
multitude of mayonnaise types, but it is also true
for other solid-liquid mixtures (see for example
the above mentioned article by H. Weisser).

The calibration and the measurement require a time
of less than five minutes per sample, therefore we
are dealing here with a fast method.

Other fast methods (e.g. the Foss-Let-Method;
A.Montag in Deutsche Lebensmittelrundschau, Volume

69, pages 470 ff., 1973: "Zur densitometrischen Fettbestimmung mit dem Foss-Let-Gerät") require at least 15 minutes and moreover use chemicals which are far more hazardous.

5

The intention of the figure is to show how the chemical drying procedure as a function of time is reflected in the spin-echo amplitude (Fig. 1) and how for a number of calibration substances the spin

10 echo amplitude (in the dried state) depends on the fat content (Fig. 2).

In detail, Fig. 1 shows the time dependence of the spin-echo amplitude after mixing with CaO for a

15 typical mayonnaise. A spin-echo sequence with 90° excitation pulse, a delay time of three milliseconds and a 90° phase shifted 180° pulse were used so that the spin echo occurred six milliseconds after the 90° pulse. For every

20 measuring point shown the sequence was repeated three times and accumulated.

At first the amplitude decreases quickly and noticeably, i.e. water is extracted and converted

25 together with the CaO into $Ca(OH)_2$. Now this part can no longer contribute to the signal. However, it can be seen that already after one minute the drying procedure is completed insofar as it does not effect a further decrease in the signal, i.e.

30 the water concentration has decreased so far that the water which may not yet have reacted with the CaO is bound in the mayonnaise in such a way that because of its short T_2 time it cannot yield a noticeable contribution to the spin echo (after six

35 milliseconds).

Fig. 2 shows the amplitude of the spin-echo signal
for three calibration mayonnaises with   fat
contents 20%, 50% and 80%,   standardized
to   one gram of mayonnaise. The three

5   measuring points lie very well on a straight line,
which, however, does not exactly go through the
origin. Therefore, in the range from 20% to 80% one
can simply interpolate linearly. On the other hand,
it is obvious that for smaller concentrations, the

10   calibration   measurement   cannot   simply   be
extrapolated linearly. Either one has to restrict
oneself to the explicit range covered or the
calibration has to be completed by additional
calibration points in order to describe the non-

15   linear range to a sufficient extent.

Claims

1. A method for determining the fat or oil content in a
sample with a high water content by means of a NMR pulse
5   spectrometer, wherein the substance is dried prior to
measurement by mixing it with a chemical drying agent.

2. A method as claimed in Claim 1, wherein the chemical
drying agent is calcium carbide.
10

3. A method as claimed in Claim 1, wherein the chemical
drying agent is CaO.

4. A method as claimed in any one of the preceding claims,
15 wherein the NMR spectrometer has a maximum proton resonance
frequency of 50 MHz and a magnetic field inhomogeneity
across the measuring volume of at least 10^{-5}.

5. A method as claimed in any one of the preceding claims,
20 wherein the NMR spectrometer is used to measure a proton
spin echo signal of the sample.

6. A method as claimed in Claim 5, wherein the proton
spin-echo signal of at least one calibration substance with
25 known fat or oil content is measured prior to the
measurement of the spin-echo signal of the sample and the
fat or oil content of the sample is derived by comparison
of the spin-echo amplitudes of calibration and sample
measurement.
30

7. A method as claimed in any one of the preceding claims,
comprising the steps of:-

    a. mixing a known amount of a calibration substance
35 of known fat or oil content with an amount of a chemical
drying agent sufficient to reduce the water content of the

calibration substance below a desired threshold,

    b.   carrying out a nuclear resonance measurement of
the reacted mixture;

5

    c.   determining the amplitude of the nuclear resonance
signal;

    d.   standardizing this amplitude to the amount of the
10 calibration substance;

    e.   recording the value of the standardized amplitude;

    f.   optionally, repeating the steps a. to e. for a
15 plurality of calibration substances;

    g.   computing a calibration table or a calibration
curve from the standardized amplitudes of step e., which
give the correspondence between fat or oil content and
20 standardized amplitude of the nuclear resonance signal;

    h.   repeating steps a. to e. replacing the calibration
substance by a sample of unknown fat or oil content;

25   i.   computing the fat or oil concentration,
respectively of the sample, from the measured standardized
amplitude of the nuclear resonance signal and the
calibration table or calibration curve, respectively;

30   j.   displaying or storing the fat or oil
concentration, respectively, for the sample.

    k.   optionally, repeating the steps h. to j. for a
plurality of samples;

35

8. A method as claimed in any one of the preceding claims,

wherein the sample is a mayonnaise.

9.   A method of determining fat content of a sample, substantially as hereinbefore described.

Patents Act 1977
.aminer's report to the Comptroller under
Section 17 (The Search Report)

Application number
GB 9219928.0

Relevant Technical fields	Search Examiner
(i) UK Cl (Edition L) G1N; G1B (BCH)	K SYLVAN
(ii) Int Cl (Edition 5) G01R (33/46)	

Databases (see over)	Date of Search
(i) UK Patent Office	
(ii) ONLINE DATABASES: WPI	17 DECEMBER 1992

Documents considered relevant following a search in respect of claims 1 TO 9

Category (see over)	Identity of document and relevant passages	Relevant to claim(s)
A	GB 2179747 A (PICKER) see whole document	–

SF2(p)

SJJ – doc99\fi1001231

Category	Identity of document and relevant passages	Relevant to claim(s.

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